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Detection of Tyrosine on Paper Chromatograms

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TYROSINE may be specifically detected on paper chromatograms of completely acid-hydrolyzed proteins by using an adaptation of Boute's [Boute, J., *Ann. endocrinol.* (*Paris*) **14**, 518–31 (1953)] method for the quantitative estimation of estrogens.

Brief exposure of the finished chromatogram to the brown vapors generated by the action of nitric acid on metallic copper, followed by exposure of the paper to ammonia fumes, gives a yellow spot with tyrosine. The color fades in air, but it can be renewed by re-exposure to ammonia fumes. The test will detect 10 γ per sq. cm. This is approximately four times the amount of amino acid required for a specific test based on the reaction of tyrosine with 1-nitroso-2-naphthol [Archer, R., Crocker, C., *Biochim. et Biophys. Acta* **9**, 704–5 (1952)]. However, the use of this reagent renders the chromatogram unsuitable for further analysis by overspraying with other reagents.

By using the method described here no background colors are obtained. The chromatogram may be oversprayed with ninhydrin or other amino acid reagents, after sufficient time has been allowed for dissipation of the gaseous reagents from the paper. Only solvents of the phenolic type interfere with the test and must be completely removed from the paper before gassing is attempted. An extensive study of the problem of removing phenolic types of compounds from paper has not been made. The last traces of phenol on air-dried paper strips can be removed without disturbing the tyrosine spot by washing the paper with ether that is undergoing continuous extraction with an aqueous solution of a strong inorganic base.

All other naturally occurring amino acids, with the exception of tryptophan, do not react. Tryptophan gives a weak test. The tyrosine derivatives 3,5-diiodotyrosine, glycyltyrosine, tyrosine ethyl ester, and tyramine, all give strong positive reactions.